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Julie Beenken,
Chemistry and Biochemistry Student,
University of Minnesota Duluth

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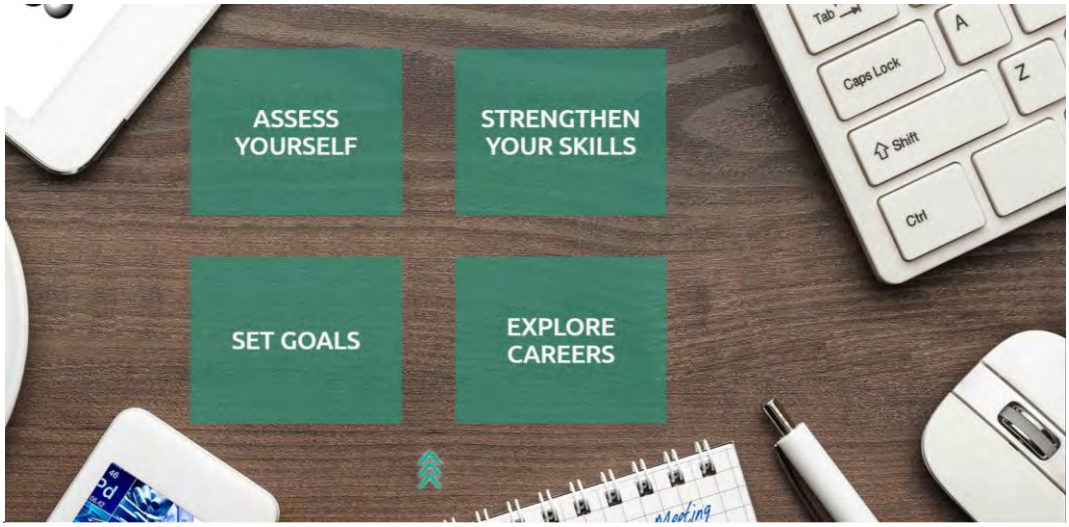
An individual development planning tool for you!



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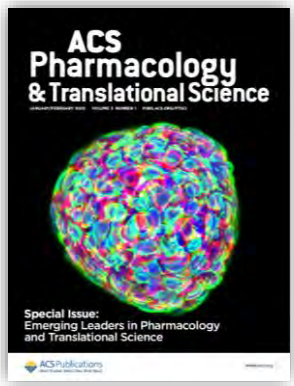

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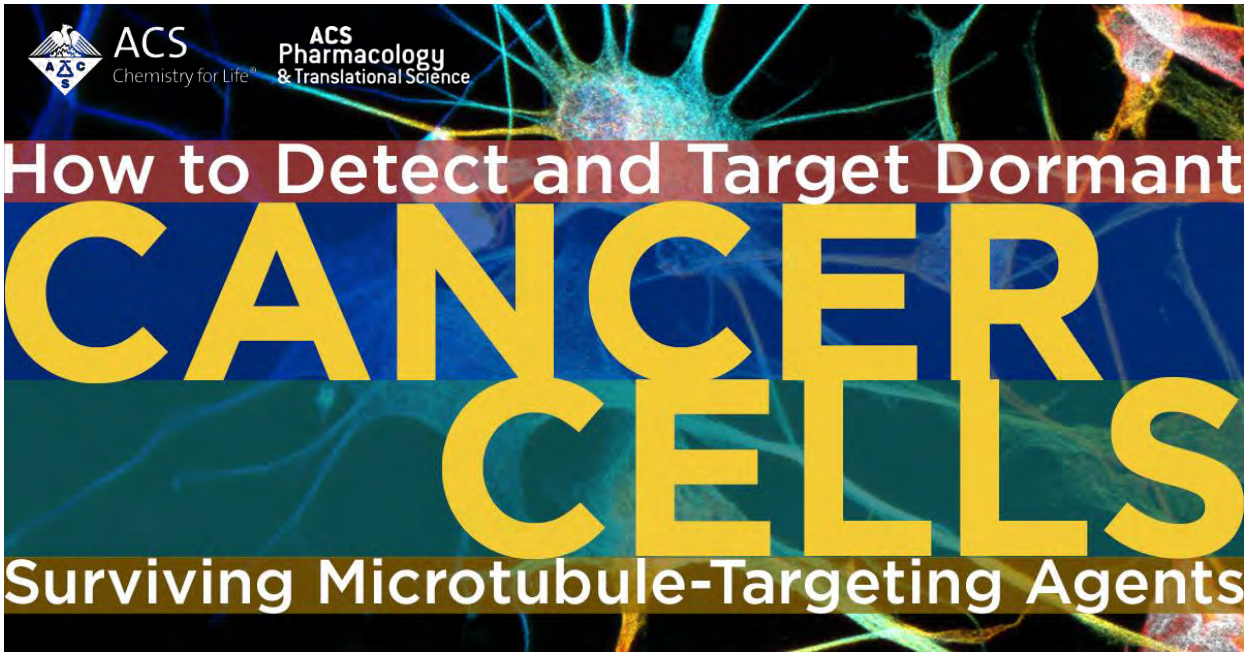

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How to Detect and Target Dormant Cancer Cells Surviving Microtubule-Targeting Agents

Patrick Sexton
Professor of Pharmacology and National Health and Medical Research Council (NHMRC) Senior Principal Research Fellow, Monash University, and Editor-in-Chief, *ACS Pharmacology & Translational Science*

Lenka Munoz
Associate Professor, Faculty of Medicine and Health, The University of Sydney

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How To Detect and Target Dormant Cancer Cells Surviving Microtubule-Targeting Agents

Presenter: Lenka Munoz, University of Sydney

Moderator: Patrick Sexton, Monash University

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What You Will Learn

- A background to the tubulin code and its impacts on the efficacy of microtubule-targeting agents
- The importance of using orthogonal inhibitors and per-division growth rate inhibition assays in cancer drug discovery
- How to detect and target dormant cancer cells

Webinar Outline

- Microtubules, tubulin code and microtubule-targeting agents
- From kinase inhibitors to microtubule-targeting agents
- Tubulin code, microtubule-targeting agents and glioblastoma
- Per-division growth rate inhibition assays in cancer drug discovery
- Cancer dormancy and drug-tolerant persister cells

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

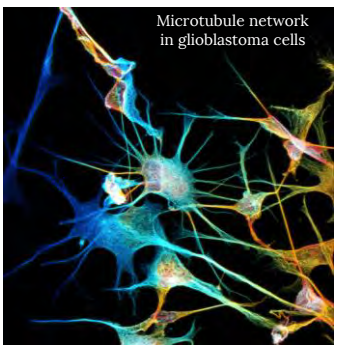
Have you worked with cells and/or analyzed cell-based data of drugs?

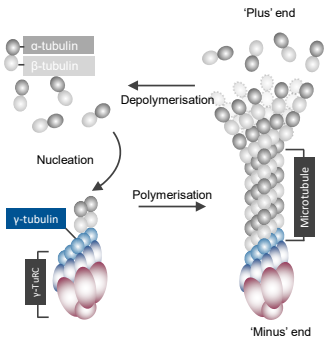
- Never worked with cells and not familiar with cell-based data
- Never worked with cells but familiar with cell-based data
- Worked with cells and familiar with analyzing cell-based data

** If your answer differs greatly from the choices above tell us in the chat!*

Microtubules

- Largest filamentous components of the eukaryotic cytoskeleton
- Essential for every cell as they control cell shape, division, motility and differentiation
- Dynamically assembled from heterodimers of evolutionary highly conserved α- and β-tubulin
- Microtubules function determined by interaction with microtubule-associated proteins and/or the tubulin code





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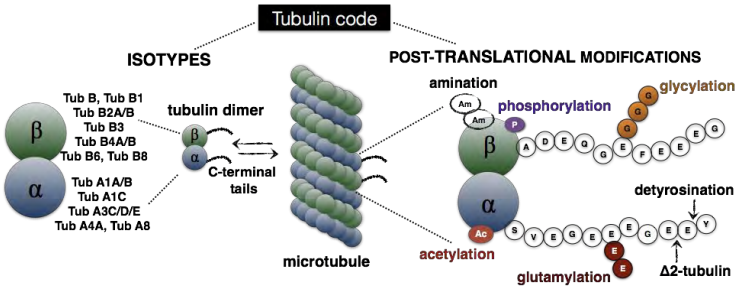
Tubulin Code

- Combination of differential expression of eight α- and nine β-tubulin genes (isotypes) and different post-translational modifications
- Impact of the tubulin code on microtubules function emerging, e.g.
  - Tubulin isotypes determine microtubule dynamics (Mol Biol Cell 2017, **28**: 3564)
  - Detyrosination of α-tubulin guides chromosomes to cell equator during mitosis (Science 2015, **348**: 799)
  - Glutamylation controls activity of microtubule severing enzymes spastin and katanin (Cell 2016, **164**: 911)
  - Phosphorylation of β-tubulin inhibits tubulin polymerization and affects dendrite morphology (Neuron 2016, **90**: 551)
- Less is known about the impact of the tubulin code on microtubule-targeting agents



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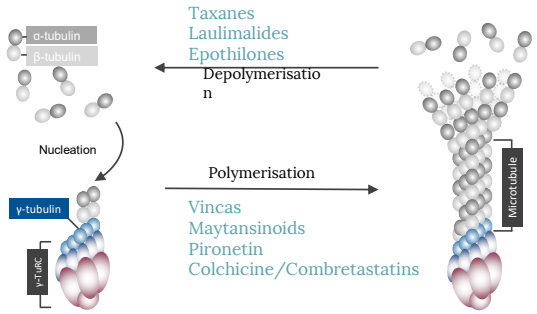
Microtubule-Targeting Agents

- Because microtubules are essential for cell division (*and cancer is primarily a hyper-proliferative disease*), microtubule-targeting agents are among the most important cancer drugs
- FDA-approved: Vincristine (1963), Vinblastine (1965), Paclitaxel (1992), Vinorelbine (1994), Docetaxel (1996), Ixabepilone (2007), Cabazitaxel (2010), Eribulin (2010)
- Non-targeted chemotherapeutics that disrupt microtubule dynamics, thereby affecting cell viability
- 6 binding sites: taxane, laulimalide/peloruside, vinca, maytasine, pironetin and colchicine



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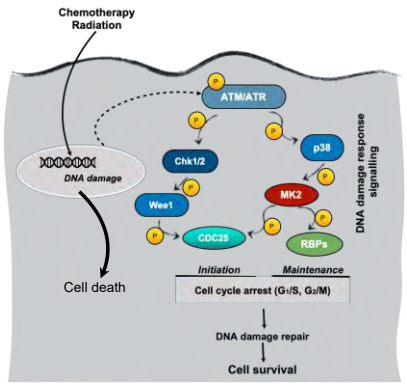
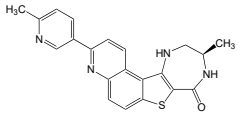
From Kinases to Microtubules

Q: Will MK2 inhibition improve chemotherapy efficacy in glioblastoma?

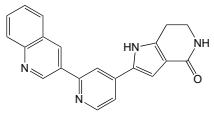


Cancer Cell 2007, **11**: 175
Mol Cell 2010, **40**: 34
Cancer Cell 2015, **28**: 623

ATP-competitive inhibitors

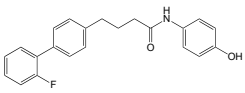


PF3644022 (Pfizer)
JPET 2010, **333**: 797
IC_{50} = 5.2 nM

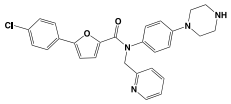


MK2 inhibitor III (Pfizer)
J Med Chem 2007, **50**: 2647
IC_{50} = 8.5 nM

Non ATP-competitive inhibitors



CMPD1 (Boehringer Ingelheim)
Biochemistry 2004, **43**: 11658
K_i = 330 nM

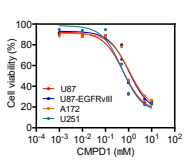


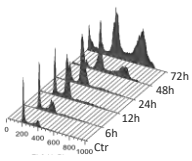
MK2 inhibitor IV (Merck)
ACS Med Chem Lett 2011, **2**: 632
IC_{50} = 110 nM

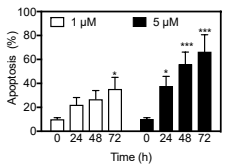
From Kinases to Microtubules

- Drug-target validation studies identified microtubules as the primary target of CMPD1
- Apoptotic efficacy of CMPD1 results from inhibiting tubulin polymerization; **not** from MK2 inhibition

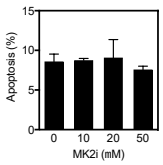
CMPD1: induced mitotic arrest and apoptosis

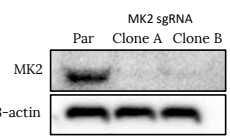


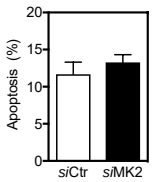


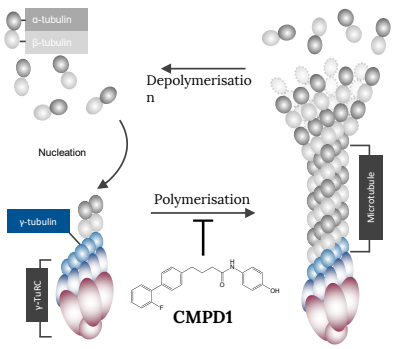


MK2 *si*RNA / sgRNA / other MK2 inhibitors: no apoptosis









Cell Death Discov 2015, **1**: 15028
Biochem Pharmacol 2015, **98**: 587
ACS Med Chem Lett 2017, **8**: 395

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

In your drug discovery research, what is your approach to off-targets in relation to the targeted protein family?

- We consider off-targets only within the targeted protein family
- We consider off-targets within and outside of the targeted protein family
- We rarely consider off-targets in our research
- Not applicable

** If your answer differs greatly from the choices above tell us in the chat!*

Non-kinase targets of kinase inhibitors

- Tubulin
  - cMet inhibitor tivantinib; CK1 inhibitor IC261; CDK4 inhibitor BTP, PLK inhibitor rigosertib
- Bromodomains
  - CDK inhibitor dinaciclib; PLK1 inhibitors BI2536 and BI6727; JAK2 inhibitor fedratinib; p38 MAPK inhibitors SB202190 and SB203580
- NQO2 enzyme is inhibited by ABL inhibitors imatinib and nilotinib
- IDOs enzymes are targets of RIPK1 inhibitor necrostatin
- Multi-kinase inhibitor sorafenib targets cystine-glutamate antiporter system in ferroptosis

Comprehensive drug-target validation includes (full guidelines in Nat Rev Drug Discov 2017, **16**: 424)

- Structurally unrelated inhibitors and structurally related but inactive analogs
- Genetic methods of target perturbation (knock-down, knock-out, drug-resistant mutation of the target)
- Correlation of activity/efficacy across orthogonal assays using cancer cell lines of varying genotypes

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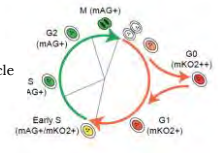
Cell morphology and cancer drugs

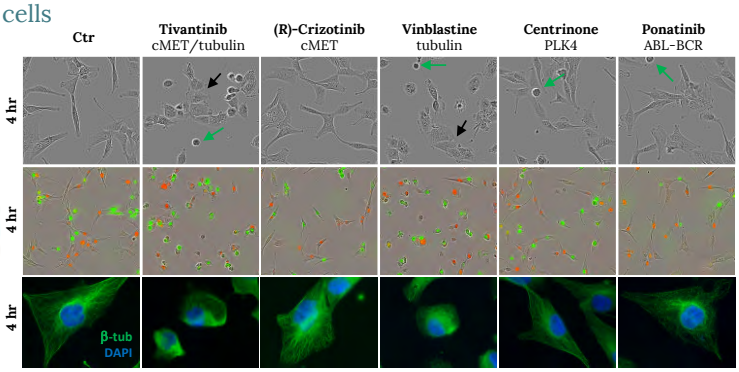
- Microtubules are essential for cell morphology
- Drugs targeting tubulin changed cell morphology within 0.5 – 4 hr => '*shrinkage effect*'
- A172 cell data confirmed with 17 cancer drugs in 7 cancer cell lines (incl. cancer stem cells)
- Early changes in cell morphology indicate tubulin as a target of kinase inhibitors

Imaging of A172 glioblastoma cells

Green arrow – Mitotic cell
Black arrow – *Shrinkage effect*

FUCCI system to monitor the cell cycle phases





Pharmacol Res 2018, **134**: 166

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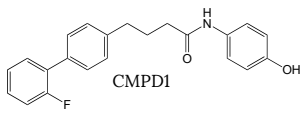
Microtubule-Targeting Agents & Brain Cancer

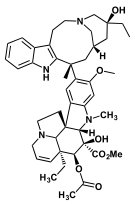
- Blood-brain barrier: major hurdle in neuro-oncology drug discovery
- CNS drugs: smaller, less polar and not a P-gp substrate
- Clinical MTAs: natural products (or analogs) with incompatible properties for BBB penetration

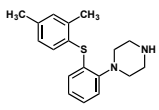
Drug Property	Kinase Inh. (n = 34)[a]	CNS drugs (n = 119)[b]	MTA (n = 8)
cLog P	4.2	2.8	2.7
TPSA	91	45	174
HBD	2	1	3
MW	483	305	768

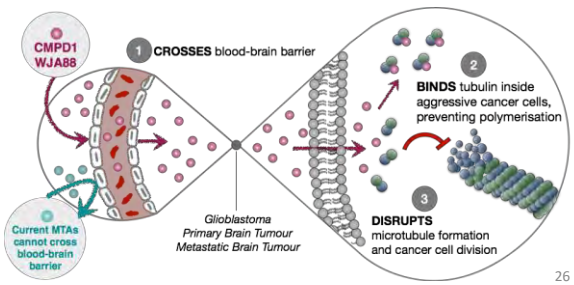
a) J Med Chem 2016, **59**: 10030
b) ACS Chem Neurosci 2010, **1**: 420



Works like vinblastine, looks like fluoxetine







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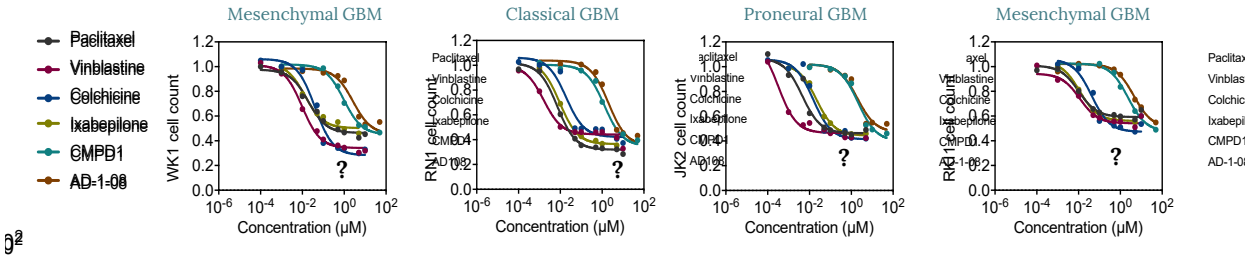
Microtubule-Targeting Agents & Glioblastoma

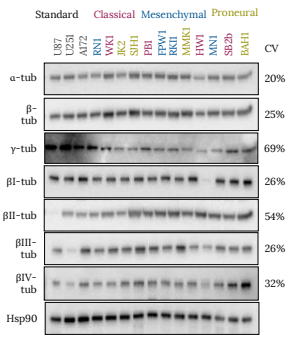
- MTAs considered *non-targeted* chemotherapeutics because microtubules expressed in all cells
- MTA efficacy is the same regardless of the MTA potency => problem is the target, not the drug
- Microtubules highly conserved in their 3D structures; but there is a significant diversity at the molecular level (tubulin code) => does tubulin code impact on MTA efficacy?

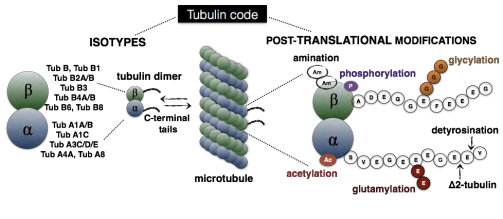


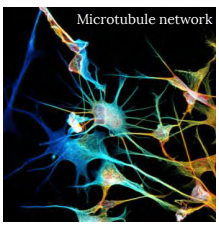
Glioblastoma (GBM) subtypes by TCGA (Cell 2013, **155**: 462)
Classical: EGFR amplification/mutation, *Ink4a/ARF* deletion Pro-neural: PDGFRA abnormalities, *IDH1* and TP53 mutations
Mesenchymal: cMET over-expression, *NF1* mutation/deletion Neural: highly differentiated phenotype

Glioblastoma & Tubulin Code

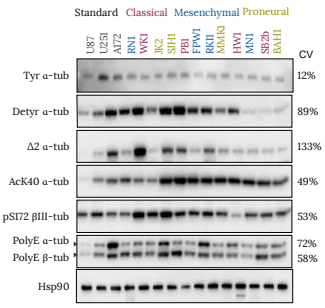
- Tubulin code of serum-grown cells (A172, U251, U87) not representative of those found in clinically relevant glioblastoma stem cells
- 20% - 130% diversity in the tubulin code within glioblastoma cell lines







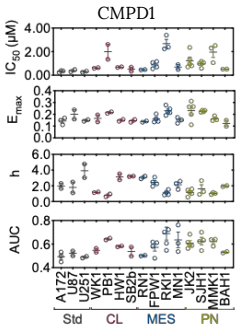
Z-Stack image of multiple β-tubulin IF images; colors assigned based on the depth of β-tubulin expression in the cell => tubulin heterogeneity within the same cell line

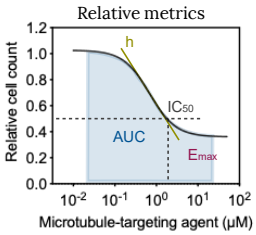


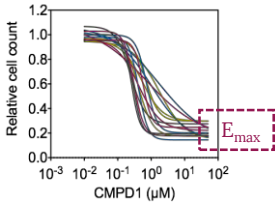
ACS Pharmacol Transl Sci 2019, **2**: 402

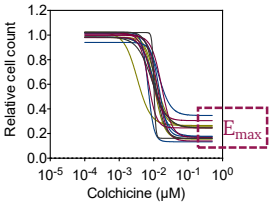
Microtubule-Targeting Agents & Glioblastoma

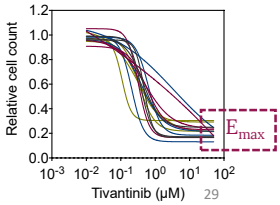
- Multiparametric analysis of dose-response curves for 6 orthogonal MTAs in 15 cell lines, 5 days viability assay
  - IC_{50} = potency
  - E_{max} = efficacy
  - h (Hill slope) = cell-to-cell variability
  - AUC = combination of IC_{50} and E_{max}
- MTA sensitivity profiles *unconvincing*
- Efficacy (E_{max}) did not fit the microscopic observations











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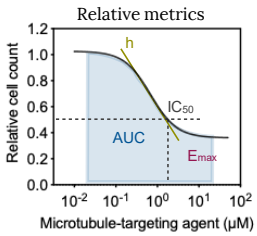
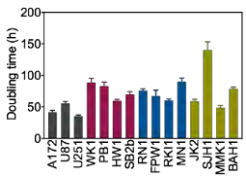
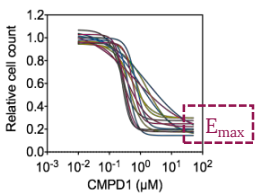
Rethinking cellular drug response

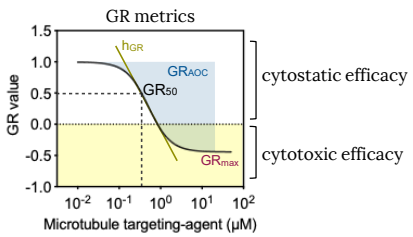
- Relative metrics confounded by the number of cell divisions taking place during viability assays
- Dependency of IC_{50} and E_{max} on division rates creates artefactual drug sensitivity (Nat Methods 2016, **13**: 521)
- Growth rate (GR) corrected dose-response curves revealed significant differences in the maximum efficacy (GR_{max})

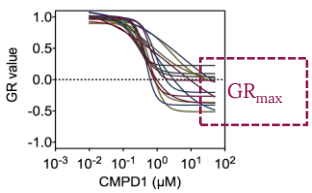






GRcalculator
Curr Protocols Chem Biol 2017, **9**: 96
Curr Protocols Chem Biol 2017, **9**: 55





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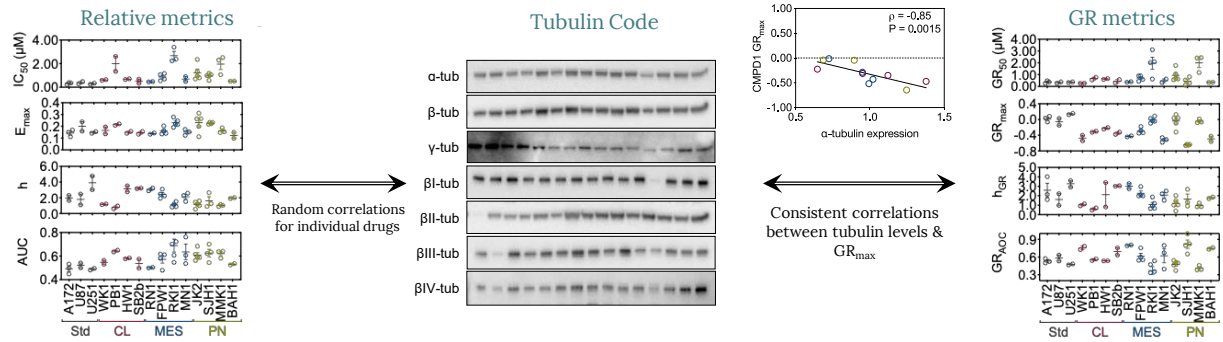
Correlating Sensitivity with Tubulin Code

- Relative metrics *vs* tubulin code => random correlations
- GR metrics *vs* tubulin code => consistent correlations between tubulin levels & efficacy
- MTA efficacy independent of tubulin isotypes and post-translational modifications
- Cells expressing less α/β-tubulin are less sensitive to MTAs



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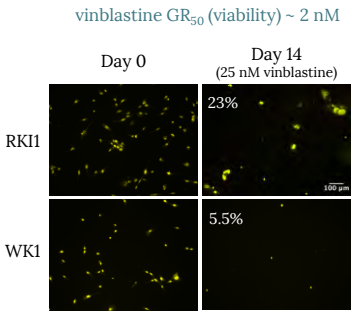
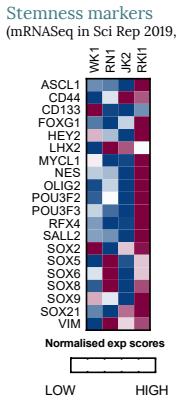
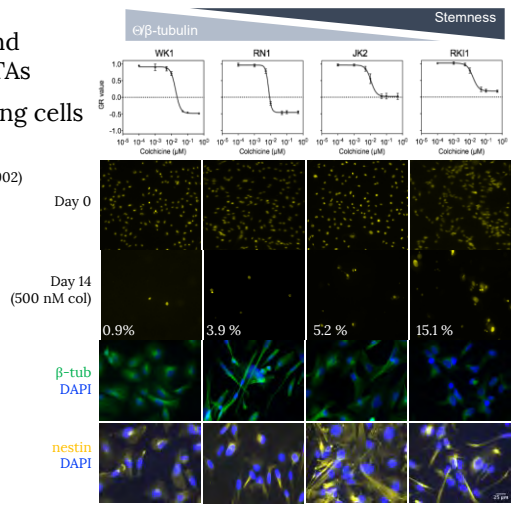
Validating the Correlations

- MTAs efficacy declines with decreasing levels of α- and β-tubulin
- Cells with less tubulin exhibit more stemness markers and survive long-term highly cytotoxic concentrations of MTAs
- Even the most potent and clinical MTAs generate surviving cells and even in sensitive cell lines







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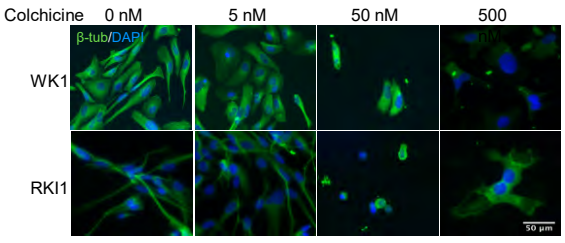
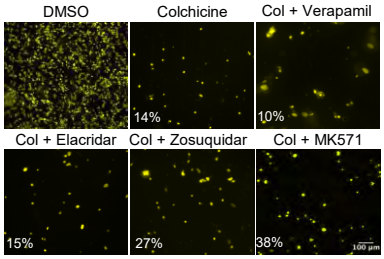
Target Engagement & Efflux Pumps

- Fractional killing could be attributed to insufficient target engagement and/or drug efflux
- Confirmed complete target engagement in all cells upon MTA treatment
- MTA efficacy independent of the expression and activity of drug efflux pumps

Target Engagement



Inhibiting Drug Efflux Pumps



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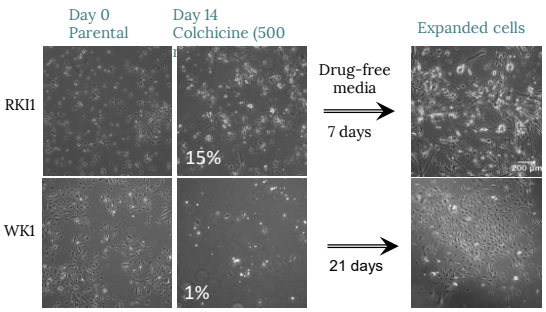
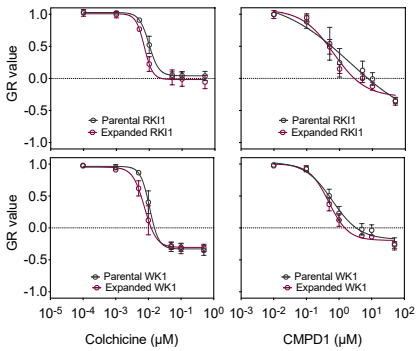
Characterization of Surviving Cells

- Surviving cells express dormancy markers: DEC2, N2RF1, p27; high p-p38/p-Erk ratio (analyzed by flow cytometry)
- Dormancy: a '*sleeping*' period in the organism's life cycle when growth, development and activity are **temporarily** stopped
- Surviving cells resume proliferation in drug-free media
- Recovered cells show equal sensitivity to MTAs => excluding drug resistance => implicating drug tolerance





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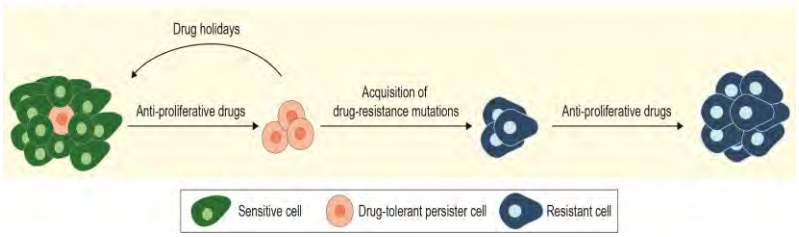
Drug Tolerance *vs* Drug Resistance

- Drug Tolerance: ability of cells to survive (but not proliferate) in the presence of cytotoxic treatments, transient non-mutational phenotype
- Drug Resistance: ability of cells to proliferate in the presence of cytotoxic treatments, irreversible (mostly mutational) phenotype
- Drug-Tolerant Persister Cells: subpopulation of cancer cells able to survive the first exposure to cancer drugs
- Drug tolerance often driven by activation of dormancy mechanisms



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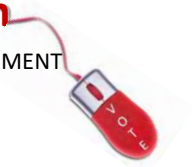
Audience Survey Question

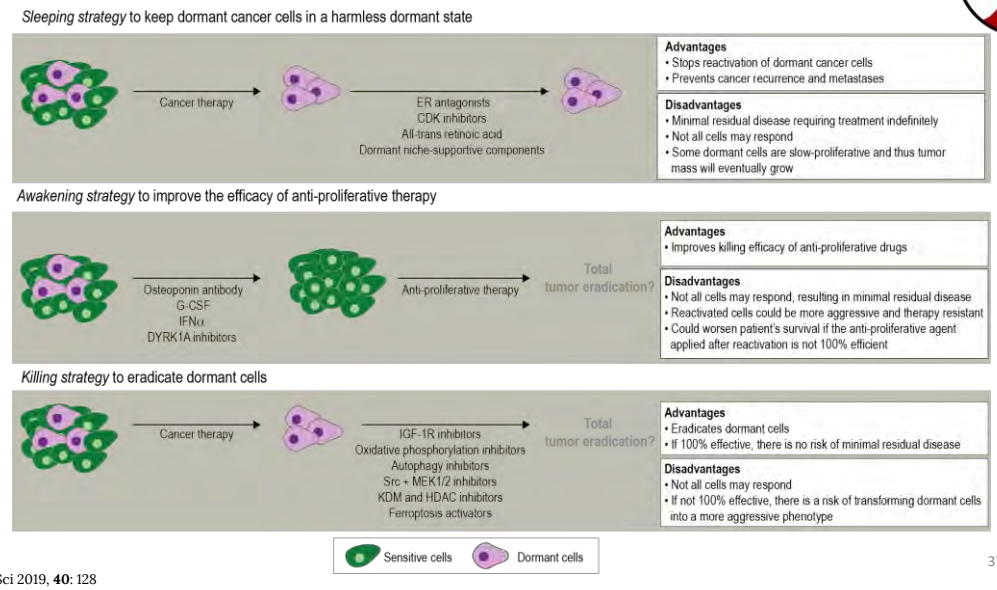
ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



Do you know from which research field originates the terminology "drug-tolerant persister cells" ?

- Neuroscience
- Microbiology
- Immunology
- None of the above

Targeting Cancer Cell Dormancy



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Summary

- Tubulin expression varies in cancer cells; MTAs follow the principal concept of pharmacology => more target = more efficacy => MTA *non-targeted chemotherapeutics??*
- Kinase inhibitors have non-kinase (off)-targets underlying/contributing to their anti-cancer efficacies => *think outside the box*
- Comprehensive drug-target validations must *never go out of style*
- Proliferation rates of cancer cells impact on drug efficacy => growth rate (GR) metrics are critical when comparing sensitivity of various cell lines
- To detect dormant cells => analyze the bottom of the dose-response curves
- Cancer is not purely a proliferative disease => dormant cancer cells detected in many cancers
- War on Cancer => War on Sleeping Cancer

Acknowledgements to all past and present members and collaborators of the Munoz Lab.

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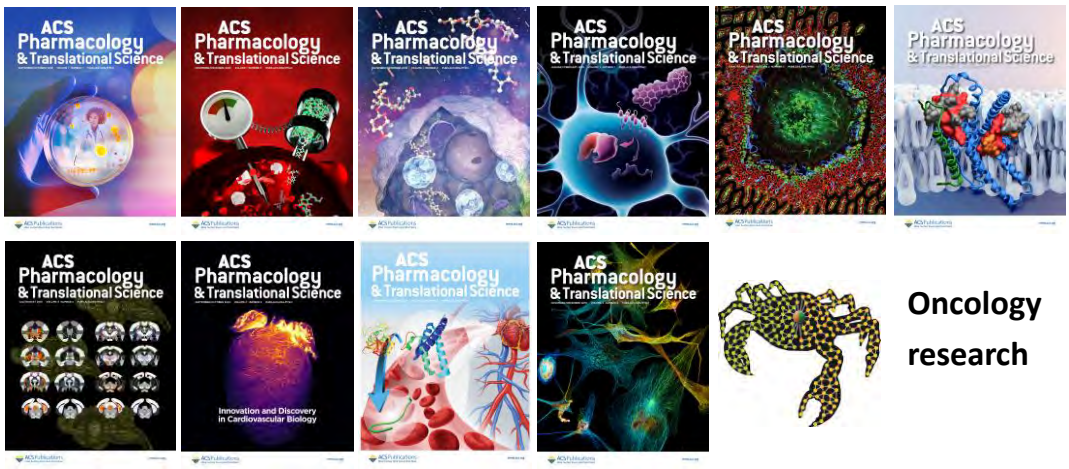

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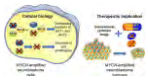
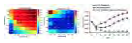


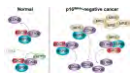
Targeting Functional Activity of AKT Has Efficacy against Aggressive Neuroblastoma

Marion Le Grand, Kathleen Kimpton, Christine C. Gana, Emanuele Valli, Jamie I. Fletcher, and Maria Kavallaris*



Synergistic Inhibition of Kinase Pathways Overcomes Resistance of Colorectal Cancer Spheroids to Cyclic Targeted Therapies

Pradip Shahi Thakuri,† Megha Gupta,† Ramila Joshi,† Sunil Singh,† and Hossein Tavana*

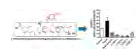


Stabilization of Cyclin-Dependent Kinase 4 by Methionyl-tRNA Synthetase in $p16^{INK4a}$-Negative Cancer

Nam Hoon Kwon,[‡,∇] Jin Young Lee,[‡,○] Ye-lim Ryu,† Chanhee Kim,† Jiwon Kong,† Seongeun Oh,† Beom Sik Kang,† Hye Won Ahn,† Sung Gwe Ahn,† Joon Jeong,† Hoi Kyoung Kim,† Jong Hyun Kim,† Dae Young Han,† Min Chul Park,† Doyeun Kim,† Ryuichi Takase,† Isao Masuda,† Ya-Ming Hou,† Sung Ill Jang,# Yoon Soo Chang,# Dong Ki Lee,† Youngeun Kim,† Ming-Wei Wang,† Basappa,† and Sunghoon Kim*[,†,⊥]

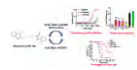
Molecular Signatures of Fusion Proteins in Cancer

Natasha S. Latysheva* and M. Madan Babu*



Aglycone Polyether Nanchangmycin and Its Homologues Exhibit Apoptotic and Antiproliferative Activities against Cancer Stem Cells

Minjian Huang,† Bo Lu,† Ran Liu,†,‡ Jian Li,† Jilei Chen,† Fenglei Jiang,† Hong Ding,† Zixin Deng,*[,†,⊥] and Tiangang Liu*[,†]

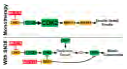


Kidney-Type Glutaminase Inhibitor Hexylselen Selectively Kills Cancer Cells via a Three-Pronged Mechanism

Jennifer Jin Ruan,[‡,▽] Yan Yu,[‡,▽] Wei Hou,[‡,▽] Zhao Chen,[‡,▽] Jinzhang Fang,† Jingjing Zhang,† Muowei Ni,† Di Li,† Shiying Lu,† Jingjing Rui,† Rui Wu,† Wei Zhang,† and Benfang Helen Ruan*[,†]

Identification of Pirin as a Molecular Target of the CCG-1423/CCG-203971 Series of Antifibrotic and Antimetastatic Compounds

Erika M. Lisabeth,† Dylan Kahl,† Indiwari Gopallawa,† Sarah E. Haynes,† Sean A. Misek,† Phillip L. Campbell,† Thomas S. Dexheimer,† Dinesh Khanna,† David A. Fox,† Xiangshu Jin,† Brent R. Martin,[†,⊥] Scott D. Larsen,[†,⊥] and Richard R. Neubig*[,†,⊥]



Differential Sensitivity to CDK2 Inhibition Discriminates the Molecular Mechanisms of CHK1 Inhibitors as Monotherapy or in Combination with the Topoisomerase I Inhibitor SN38

Nicholas J. H. Warren, Katelyn L. Donahue, and Alan Eastman*

The HPV Vaccine Story

Ian H. Frazer*

Cathepsin B Dependent Cleavage Product of Serum Amyloid A1 Identifies Patients with Chemotherapy-Related Cardiotoxicity

Fangfang Zhang,[†,⊥] Christopher J. Lyon,† Robert J. Wills,† Bo Ning,† Jia Fan,*[,†] and Tony Y. Hu*[,†,⊥]

Lower Tubulin Expression in Glioblastoma Stem Cells Attenuates Efficacy of Microtubule-Targeting Agents

Ramzi H. Abbassi,† Ariadna Recasens,† Dinesh C. Indurthi,† Terrance G. Johns,† Brett W. Stringer,‡ Bryan W. Day,‡ and Lenka Munoz*[,†]

Advances on Tumor-Targeting Delivery of Cytotoxic Proteins

Akmal M. Asrorov,[†,‡] Zeyun Gu,† Kyoung Ah Min,‡ Meong Cheol Shin,*[,‡,⊥] and Yongzhuo Huang*[,†]

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